

We claim:

1. A method of determining enzyme activity comprising:

5        contacting a compound selected from the group consisting of enzymes, enzyme fragments and abzymes with a labeled substrate to form a differentially-charged product;

10      selectively coupling either the substrate or the differentially-charged product to an ion-exchange resin thereby substantially separating the substrate from the differentially-charged product; and

15      determining the amount of substrate remaining or differentially-charged product formed using a measuring means.

- 15 2. A method of determining enzyme activity comprising:

20        contacting a compound selected from the group consisting of enzymes, enzyme fragments and abzymes with a labeled substrate thereby effecting the conversion of the substrate to a differentially-charged product;

25        stopping the conversion before all of the substrate present has been converted to the differentially-charged product;

30        selectively coupling either the substrate or the differentially-charged product to an ion-exchange resin thereby substantially separating the substrate from the differentially-charged product in a single step; and

35        determining the amount of substrate remaining or differentially-charged product formed using a measuring means;

40        wherein the stopping step and coupling step are carried out concurrently or sequentially.

3. The method of claim 1 or 2 wherein the product is bound to the resin.

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4. The method of claim 1 or 2 wherein the substrate is bound to the resin.

5. The method of claim 1 or 2 wherein the product or substrate measured is coupled to the resin

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6. The method of claim 1 or 2 wherein the product or substrate measured is in solution

7. The method of claim 1 or 2 wherein said enzyme is a kinase.

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8. The method of claim 1 or 2 wherein said method is conducted in a multiple-well format.

9. The method of claim 8 wherein the format comprises at least about 96 wells.

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10. The method of claim 8 wherein said format is automated.

11. The method of claim 1 or 2 wherein said high-throughput format is conducted on a microchip.

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12. The method of claim 1 or 2 wherein said enzyme is selected from the group consisting of GFAT, Nitric Oxide Synthase, Methionine Aminopeptidase, Asn Syn, PFK, p38, I-kappa kinase 1, I-kappa kinase 2, TBK1, MAPKAP 2, GTase, , OGTase, and Cyclooxygenase.

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13. A method for identifying a molecule, compound, or composition that affects the activity of an enzyme, comprising:

contacting the enzyme with a test sample comprising a molecule, compound, or composition;

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contacting the enzyme with a labeled substrate to form a differentially-charged product;

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- selectively coupling either the substrate or the differentially-charged product to an ion-exchange resin thereby substantially separating the substrate from the differentially-charged product;
- 5       determining the amount of substrate remaining or differentially-charged product formed using a measuring means; and
- comparing the amount of substrate remaining or differentially-charged product formed with a control.
- 10      14. The method of claim 13 wherein said enzyme is selected from the group consisting of GFAT, Nitric Oxide Synthase, Methionine Aminopeptidase, Asn Syn, PFK, p38, I-kappa kinase 1, I-kappa kinase 2, TBK1, MAPKAP 2, GTase, , OGTase, and Cyclooxygenase.
- 15      15. The method of claim 13 wherein the control is an isozyme and the method is used to identifying a compound or composition that preferentially or specifically effects an enzyme over its isozyme.
- 20      16. A method of determining bi-functional enzyme activity comprising:  
          contacting an enzyme with a first labeled substrate to form a first differentially-charged product;
- 25            contacting the enzyme with a second labeled substrate to form a second differentially-charged product;
- selectively coupling to an ion-exchange resin a member selected from the group consisting of the first substrate, the second substrate, the first product, and the second product, thereby substantially separating said member from the remaining members of the group; and
- 30            determining the amount of said member using a measuring means.
- 35      17. A method of determining bi-functional enzyme activity comprising:  
          contacting an enzyme with a first labeled substrate to form a first differentially-charged product;
- contacting the enzyme with a second labeled substrate to form a second differentially-charged product;

selectively coupling to an ion-exchange resin two members selected from the group consisting of the first substrate, the second substrate, the first product, and the second product, thereby substantially separating said members from the remaining members; and

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determining the amount of said members using a measuring means.

18. The method of claim 17 wherein said determination of bi-functional enzyme activity is conducted separately.

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19. A method of determining the kinetics of an enzyme reaction, comprising: contacting a compound selected from the group consisting of enzymes, enzyme fragments and abzymes with a labeled substrate to form a differentially-charged product;

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stopping the reaction at various timepoints;

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selectively coupling either the substrate or the differentially-charged product to an ion-exchange resin thereby substantially separating the substrate from the differentially-charged product;

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determining the amount of substrate remaining or differentially-charged product formed using a measuring means; and

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comparing the amount of substrate remaining or product formed at the timepoints.

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20. A method of determining the functional sites on an enzyme comprising : contacting a compound with a plurality of point-mutated enzymes with a labeled substrate to form a differentially-charged product;

selectively coupling either the substrate or the differentially-charged product to an ion-exchange resin thereby substantially separating the substrate from the differentially-charged product;

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determining the amount of substrate remaining or differentially-charged product formed using a measuring means; and

comparing the amount of substrate remaining or differentially-charged product formed from the plurality of enzymes.

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21. A method of evaluating the selective coupling of an enzyme and a reactant comprising contacting a compound with an enzyme with a plurality of labeled substrates to form differentially-charged products;

5 selectively coupling either the substrates or the differentially-charged products to an ion-exchange resin thereby substantially separating the substrates from the differentially-charged products;

10 determining the amount of substrate remaining or differentially-charged products formed using a measuring means; and

comparing the amount of substrate remaining or differentially-charged products formed from the plurality of substrates.

15 22. A kit for determining enzyme activity wherein said kit comprises at least three members of the group consisting of: An enzyme, a labeled ligand, a buffer solution, an ion-exchange resin, and a stop-buffer solution

20 23. A kit of claim 22 for determining enzyme activity wherein said kit comprises at least three members of the group consisting of: An enzyme, a labeled ligand, a buffer solution, an ion-exchange resin, and a stop-buffer solution

25 24. A kit of claim 23 for determining enzyme activity wherein said kit comprises at least three members of the group consisting of: An enzyme, a labeled ligand, a buffer solution, an ion-exchange resin, and a stop-buffer solution

26. A kit for determining enzyme activity comprising an enzyme, a labeled ligand, a buffer solution, an ion-exchange resin, and a stop-buffer solution

30 26. A compound discovered using the method of claims 1 or 2.